



# The Effect of Innovation in the Culinary Processing of Bitter Melon (*Momordica charantia*) on Wistar Rats' Change in Glucose Level (*Rattus norvegicus*)

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This research was aimed at investigating the innovation effect of processing bitter melon fruit culinary on the change of glucose level of diabetic rats. Hyperglycaemia of the rats' condition was done with induced Streptozotocin (STZ) using the change of glucose level test for a 4-week experiment. Another test that was done was a glucose response test and a short-chain fatty acid test (SCFAs). The 30 rats were divided into 5 experimental groups: group 1 belonged to normal rats and this was the control group; group II was rats with hyperglycaemia; groups III, IV and V were hyperglycaemic rats with a diet of boiling bitter melon, steaming bitter melon and sautéing bitter melon. The research findings showed that giving a diet of bitter melon with boiling treatment, steaming and sautéing, caused a significant ( $p < 0.05$ ) change in the rats' glucose level, SCFAs profile and inhibition of glucose absorption. Creativity in bitter melon culinary processing produced sautéed bitter melon that inhibited glucose absorption greater than steamed and boiled bitter melon.



SCFAs profile produced were acetic acid > propionate acid > butyrate acid. This fact can prevent coronary heart disease.

**Keywords:** *Innovation processing, Momordica charantia, Change of glucose level.*

## INTRODUCTION

*Diabetes Mellitus* (DM) is a chronic disease caused by a *heredity* factor, that is, the lack of insulin secretion by the pancreas or by the ineffectiveness of insulin produced due to the damage of insulin activity. This problem increases the glucose concentration in the blood that can damage the nervous system, especially blood arteries and nerves (Prabhakar et al., 2011). The presence of Hypoglycaemia was signalled by the increase of glucose level in blood serum which exceeded 200 mg/dL, on the condition of no fasting (Niu et al., 2010). Diabetes mellitus is a complicated metabolic disease and affects more or less 5% of people in the world. *Diabetes mellitus* was divided into 2 types: type I and type II. Type I *Diabetes* was 5-10% from all diabetic cases and came from autoimmune pancreas  $\beta$ -cell damage that grew rapidly, caused by serious cell damage. This took out insulin so that the patients depended on being given insulin. Type II diabetes often occurred covering 90-95% from all cases signaled by insulin resistance and was often accompanied by obesity. Modern people often consumed more food and did less physical exercise, which dramatically increased the irregular metabolism problem of *diabetes mellitus*.

*Momordicacharantia*, Linn was one of the food materials having potential to be developed in Indonesian culinary arts. *Momordicacharantia*, Linn in this age was used widely to manage diabetes and its complications (Kumar et al., 2011). The component inside *Momordica charantia*, having a role in decreasing blood glucose level is pectin and dietary fibre. Pectin is one of the soluble dietary fibres having a physiological effect in decreasing blood glucose levels through a fermentation process mechanism in digestion, so that it builds gas and short chain fatty acids that could be expelled from body. Through this mechanism, carbohydrate metabolism inside the body can be improved so that it decreases the blood glucose levels of diabetic rats. Dietary fibre, especially soluble dietary fibre, decreases blood glucose levels by decreasing postprandial glucose caused by the characteristics which can make gel structures



and thick solutions (Weickert et al., 2008). Food with high soluble fibre levels was recommended for diabetic patients because the thick characteristic of fibres and its gel formation, inhibited macronutrient absorption.

Food material in Indonesia is not consumed in the form of raw material as it is processed into various foods. The cooking of food is used for preservation so that the cooked food can be saved longer in the appropriate cool conditions, compared with raw materials (Estiasih et al., 2009). This is the innovation of how the Indonesian people processed their food. Almost all-Indonesian traditional foods were processed using heating until cooked. It was especially useful for processing vegetables as often vegetables were washed, squeezed and salt was added, and the water was thrown away before seasoning was added and served. By using this method, some components were thrown away through washing out, and some of the non-heat resistant components were damaged during the food cooking process. The change of ways in cooking fresh bitter melon as a vegetable fruit, which is often consumed by the Indonesian community, is through steaming, boiling and sautéing processes.

This research aimed to test the innovation treatment effect of the bitter melon culinary processes of *steaming*, *boiling*, and *sautéing* on diabetic rats, (*Rattus norvegicus*) induced streptozotocin (STZ). Streptozotocin (STZ), which was combined using nicotinamide (NA)STZ caused the damage of  $\beta$ -cell pancreas because it lacked  $\beta$ -cell mass and the existence of metabolic damage on the cell which took out insulin; while NA was used to protect the cells partially, which took out insulin towards STZ. The level of serious experimental diabetic rats depended on the STZ doses and induced NA (Szkudelski, 2012).

## **MATERIALS AND METHODS**

### ***MATERIALS***

Fresh *bitter melon* material was obtained from a local farmer in East Jawa. Other materials used were an AIN 93 M standard diet, streptozotocin (STZ) and nicotinamide (NA) taken from Food and Nutritious Laboratory, Central University Connection (PAU), Yogyakarta Gajah Mada University. Reagent analytical grade such as 90% Methanol (pa) and n-hexane, DiaSys



(Diagnostic System) Glucose GOD FS, Na-phosphate buffer (pH 7.0) were achieved from *Sigma Chemical Co.*

### ***DESIGN EXPERIMENT***

The research subjects of 30 male rats (*Rattusnorvegicus*) were placed in a hollow stainless-steel cage individually. Then, they were conditioned in the cage for a week for adaptation. Finally, they were divided into 5 treatment groups:

1. normal rats with a standard AIN 93M diet;
2. diabetic rats with a standard AIN 93M diet;
3. diabetic rats with a diet of boiled bitter melon;
4. diabetic rats with a diet of steamed bitter melon;
5. diabetic rats with a diet of sautéed bitter melon.

### **HYPOGLYCAEMIC ACTIVITY TEST**

The research had been approved by good ethics commission Animal Care and Use committee, Brawijaya University, Indonesia, with the number of 467-KEP-UB 2015. The rats' experiment was in an hyperglycaemic condition using induced Streptozotocin (STZ) and NA through intraperitoneal injection of 65 mg/kg rats' body weight (Szkudelski, 2012). After 5 days, it was found that the rats' experiment experienced an hyperglycaemic condition indicated by blood glucose levels of more than 180 mg/dL. Injected STZ and NA was given to all experimental rats groups except the control group (normal rats). Besides the treatment of bitter melon food, rats were given an AIN 93M of a standard diet (Reeves et al, 1993). Giving a standard diet was done *ad libitum* while giving the diet food of bitter melon was force fed. The research was done for four weeks with the parameter of blood glucose level, the rats' body weight, and the residual feed was done at day 0, 7, 14, 21, and 28. At the end of the research, treatment was done taking caecum digesta rats and initiated anaesthesia with ether. The blood sample needed was taken from retro-orbital plexus and the glucose level was measured using GOD/PAP method (Zhao et al, 2007). A caecum digesta was done, as well as the pH measurement and SCFA (Short Chain Fatty Acids) analysis using chromatography method (Henningsson et al., 2002).

## **STATISTICAL ANALYSIS**

Research about testing blood glucose was designed using *Nested Design* method, consisting of two factors. The result of the data was analysed using analysis of covariance (ANOVA) and continued with DMRT (Duncan's Multiple Range Test).

## **GLUCOSE RESPONSE TEST**

The Glucose Response Test done referred to research conducted by Xie et al. (2004) with some modification. Twelve rats were adapted using AIN 93-M diet for three days and divided into four groups:

Group 1: controlled rats with AIN 93M diet standard diet.

Group 2: rats with steamed bitter melon diet.

Group 3: rats with boiled bitter melon diet.

Group 4: rats with sautéed bitter melon diet.

Every rat was fed with glucose of 2 grams/kg body weight doses and given pulp of bitter melon treatment with as much as 200 mg/kg body's weight. Before taking blood to measure glucose level, rats were fasted for 16 hours. Measurements of the blood sample levels taken from retro-orbital plexus glucose was analysed in the minutes of 0, 30, 60, 90, and 120.

## **RESULTS**

Protein component content, fat, total sugar and total carbohydrate were presented in figure 1 while the dietary fibre content of soluble dietary, insoluble dietary, total dietary fibre content and pectin content of bitter melon were presented in figure 2. Component content and dietary fibre content of figure 1 and 2 were bitter melon content given treatments of boiling, steaming and sautéing.

The dietary fibre content of bitter melon on three treatments was higher than insoluble fibre content and the pectin content was lower than the soluble fibre. However, it was still higher than insoluble fibre content. The protein component content was important relating to the dietary fibre content because of the browning reaction in steaming and sautéing bitter melon.

Bitter melon fat content sautéed was the highest among the boiling and steaming bitter melon. The effect of giving a diet of bitter melon with boiling, steaming and sautéing on rats body's weight and pH digesta were presented in table 1.

In table 1, it was found that food consumption, rats' body weight, and pH digesta of the rats' experiment were significantly different ( $p < 0.05$ ). Giving AIN93M standard diet, diet of boiling, steaming and sautéing treatment towards SCFAs digesta concentration, consisting of acetate acid, propionate acid and butyrate acid on the group of rats, were presented in figure 2. The changes of the rats' blood glucose level for a four-week experiment by giving diet food of boiling, steaming and sautéing treatment were presented in table 2 and figure 4.

Table 2 showed that giving diet of bitter melon with boiling, steaming and sautéing, could decrease the rats' blood glucose level significantly ( $p < 0.05$ ). The glucose response test was done to find out the effect of glucose absorption inhibition of blood, after the process of glucose gastric intestine. The result of the rats' blood glucose response test with diet that was boiled, steamed and sautéed were discussed in figure 4.

## DISCUSSION

Cellulose, hemicelluloses, non-carbohydrate lignin component, pectin substance, gum and mucilage were the dietary fibre component of the important plant in the diet and could not be digested in the process of enzymatic gastric intestine. Cellulose, hemicelluloses and lignin belonged to insoluble dietary fibre (water insoluble/less fermented fibres), while pectin, gums and mucilage belonged to water soluble/well fermented (Blackwood et al). Pectin was a substance group of complicated polysaccharide with the main component of D-galakturonat acid as compiler component of plant wall function as a substance that strengthens intercellular. Pectin had the highest water-soluble fibre and could be metabolised by a perfect bacteria colony. Due to the characteristic ability in forming gel, soluble dietary fibre could decrease velocity or pending empty gastro as well as affected towards transit time in the small intestine. This characteristic was very important in explaining hypoglycaemic mechanism (Dhingra et al., 2012).



The research revealed that the bitter melon using boiling, steaming and sautéing treatment had a higher soluble dietary fibre content compared with insoluble dietary fibre content, as well as its pectin content. Soluble dietary fibre had an important role in decreasing rats' blood glucose level through the mechanism of viscosity and its nature to form gel so that it could trap glucose and macronutrients causing the slowness of its absorption process (Nugent, 2005).

The protein content of bitter melon with steaming treatment ( $(15 \pm 0.8)$  %) and sautéing treatment ( $(2 \pm 0.9)$  %) produced in this research was lower compared with boiled bitter melon ( $(29 \pm 1.5)$  %). It could be explained that the mechanism that steamed and sautéed bitter melon reacted to the browning. The browning reaction was caused from the reaction between protein and carbohydrate inside the bitter melon during thermal processing in the cooking method. Cooking could decrease contaminant microorganisms and inactively unwanted enzymes, including polifenolase that could catalyse oxidation reaction towards phenol compound, causing formation of brown colour because of browning reaction. The cooking process using the boiling method that could inactivate the polifenolase enzyme was steaming and sautéing cooking methods, so that the most widely browning reaction was the sautéing method cooking process. Besides, the polifenolase enzyme activity, browning reaction was also caused by the reaction of protein and carbohydrate. Bitter melon cooked using the sautéed method produced the lowest protein content compared to the steamed and boiled bitter melon protein content. Cooking using the sautéed method could decrease component losses of food material because of the dissolution process.

The existence of the thermal process in the cooking method and the occurrence of browning reaction in the cooking method of steaming and sautéing, caused dietary fibre content of steamed bitter melon ( $(47.7 \pm 0.8)$  %) and sautéed ( $(49.1 \pm 0.2)$  %) higher than boiled bitter melon ( $(39.2 \pm 0.8)$ ). This was what the researcher did before (Björck et al., 1984; Váró et al., 1983), stating that the thermal process caused the reduced small amount of starch by the enzyme ability and increased dietary fibre content value. If the food component could not be digested, it could be considered as a dietary fibre component. This mechanism was as a resistant starch product produced from the thermal process or dehydration process, providing more structure on the starch molecule so it could not be digested by the enzyme.

The normal rats' body weight decreased after it was induced with streptozotocin (STZ). It showed one of the successful indicators of the male rat diabetic experiment (Al-Shamaony et al., 1994). The research showed that the consumption after the process of using boiling, steaming and sautéing method, affected significantly ( $p < 0.05$ ) towards the change of rats' body weight. According to Chen et al. (1982), the decrease of the hyperglycaemic rats' body weight happening, was likely to be the effect of protein reduction caused by the unavailability of carbohydrate to be used as an energy source. The processed bitter melon in this research with high enough carbohydrate content about 42.9% - 51.2% possibly functioned as one of the carbohydrate sources for energy and could decrease protein waste until it increased the rats' body weight. The increased food consumption on hyperglycemic rats compared with normal rats possibly also gave an additional energy source.

Figure 2 exposed that SCFA's concentration was highest in normal rats compared with other SCFAs concentration on the rats' treatment. This SCFA's concentration decreased high enough on the hyperglycaemic rats' group until it was on the lowest concentration position, compared with the other treatment. The low SCFA's concentration on the hyperglycaemic rats' group increased with giving treatment of the processed bitter melon diet using boiling, steaming or sautéing methods. The increase of SCFA's concentration is on giving treatment of the processed bitter melon diet, boiled, steamed and sautéed, related to a digesta pH decrease as shown in table 1. The rats' group given sautéed diet bitter melon produced the highest digesta SCFAs, followed by the steamed diet bitter melon rats' group, and the lowest SCFA's concentration was on the boiled diet bitter melon rats' group. Rats' digest SCFA's Profile on sautéed and steamed diet bitter melon successively were  $146 \pm 6\%$  and  $115 \pm 7\%$  acetate acid, and  $51 \pm 8\%$  and  $44 \pm 4\%$  propionate acid. The sautéed diet bitter melon rats' group produced digesta with concentration of ( $22 \pm 4\%$ ) of highest butyrate acid. The high butyrate acid concentration had good potential to protect colon cancer.

Type's variety and SCFA's concentration produced, were affected by fermented polysaccharide's type in the intestine, related to its physical and chemical properties. Soluble dietary fibre negated absorption in the small intestine until fermentation was done in the colon by the bacterial colony and produced SCFAs. Metabolised SCFAs' type by mucosa colony especially, was butyrate acid, while propionate acid and acetate acid would be absorbed

accurately (Theuwissen et al., 2008). The fermentation process in the colon would break more than 75% dietary fibre on a diet producing carbon dioxide production, hydrogen, metan and SCFAs (Slavin et al, 2004). SCFA's digesta profile on the rats' treatment group with boiled, steamed and sautéed diet bitter melon on this research successively, were acetate acid concentration, propionate acid > butyrate acid. Acetate acid and propionate would be absorbed quickly by hepatic blood vein and had a role in decreasing blood glucose level and protecting insulin resistance (Henningsson et al., 2002).

Hypoglycaemic activity was shown by the decrease of hyperglycaemic rats' blood glucose level after consuming boiled, steamed and sautéed bitter melon. 223.25 mg/dL hyperglycaemic rats' blood glucose level decreased quite sharply after consuming steamed and sautéed bitter melon successively 51.35% and 50.78%, while the decrease of the rats' blood glucose level after consuming boiled bitter melon was 48.28%. The analysis result of variance shown the rats' blood glucose level after consuming steamed bitter melon (108.60 mg/dL) and sautéed (109.88 mg/dL) were not different significantly, but for blood glucose level after consuming boiled bitter melon (115.46 mg/dL) was significantly different (significant  $p < 0.05$ ). The result occurred because the existence of dietary fibre on steamed and sautéed bitter melon, having dietary fibre content, was higher than the boiled bitter melon. Dietary fibre was viscous and formed gel formation that could trap other glucose and macronutrient so that it decreased postprandial glucose, due to the lower absorption process (Weickert et al., 2008). The glucose response test on figure 4 showed the result that giving boiled, steamed and sautéed bitter melon could inhibit glucose absorption. The glucose response test showed glucose absorption inhibition after digesting glucose inside the digestion tool system. The glucose response test result in the research showed glucose inhibition from sautéed bitter melon > steamed bitter melon > steamed bitter melon. For the control group treatment with standard diet AIN 93M, indicated the lowest glucose absorption inhibition, so that produced the highest glucose increase. The browning reaction happened in sautéed bitter melon was more compared with steamed bitter melon, causing total dietary fibre content of sautéed bitter melon to be higher than steamed and boiled bitter melon. Because of viscosity and ability in forming gel from dietary fibre, this caused the higher amount of dietary fibre in sautéed bitter melon having the highest glucose absorption inhibition, compared followed by the steamed and boiled bitter



melon. The characteristic of forming gel formation from dietary fibre was able to inhibit empty gastric and speed transit time and control nutrition absorption so that effected glucose absorption as well as food product glycaemic index (Lunn et al., 2007). Dietary fibre was able to inhibit the nutrition spread during intestinal mucosa absorption so it decreased blood glucose level.

## CONCLUSION

Bitter melon (*Momordicacharantia*, L) in general was not consumed raw, but it was often consumed in the processed form. The bitter melon innovation processing was done using boiled, steamed and sautéed methods. Consumption of diet boiled, steamed and sautéed bitter melon for four weeks, was able to decrease blood glucose level significantly ( $p < 0.05$ ). Hyperglycaemic rats consuming a diet of steamed and sautéed melon, decreased blood glucose level more sharply than rats consuming boiled diet bitter melon. The change of rats blood glucose level consuming sautéed and steamed bitter melon was higher than the rats consuming the boiled bitter melon. Steamed and sautéed bitter melon's dietary fibre content was higher than boiled bitter melon. This happened because of the existence of the browning reaction during the steamed and sautéed thermal processing. The browning reaction that occurred on sautéed bitter melon was more than steamed bitter melon, but it was not significantly different ( $p < 0.05$ ). The browning process produced an undigested component so it was included in the dietary fibre component. The possibility of the browning reaction was the Maillard reaction due to the quite high protein content and carbohydrate in steamed and sautéed bitter melon. SCFA's digesta profile, which produced a concentration composition of acetate acid > propionate acid > butyrate acid, had a significant role in decreasing blood glucose level and protecting insulin resistance. The sautéed bitter melon had ability to inhibit the highest glucose absorption followed by steamed and boiled bitter melon. Standard diet without adding bitter melon produced the increase of the highest blood glucose level as well as the lowest glucose absorption inhibition.



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### **Authors' contribution:**

All authors contributed together in conducting the research and prepared this manuscript.

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**Table 1.** Body weight, food consumption, and caecum digesta of rats with diet of bitter melon.

Treatment	Body weight /28 hr (g)	Food consumption/day (g)	pH
Normal rats 6.42 <sup>a</sup> ± 0,09	206.08 <sup>a</sup> ± 12.29	16.59 <sup>a</sup> ± 0.47	
Diabetic rats(DM) 6.90 <sup>c</sup> ± 0,02	167.50 <sup>b</sup> ± 12.57	18.95 <sup>c</sup> ± 0.19	
DM rats with diet fed :			
Boiling bitter melon 6.71 <sup>b</sup> ±0,05	183.42 <sup>c</sup> ± 14.36	17.19 <sup>b</sup> ± 0.63	
Steaming bitter melon 6.62 <sup>b</sup> ± 0,22	209.08 <sup>a</sup> ± 10.89	17.02 <sup>b</sup> ± 0.72	
Sauting bitter melon 6.65 <sup>b</sup> ± 0,09	210.54 <sup>a</sup> ± 8.15	17.03 <sup>b</sup> ± 0.56	

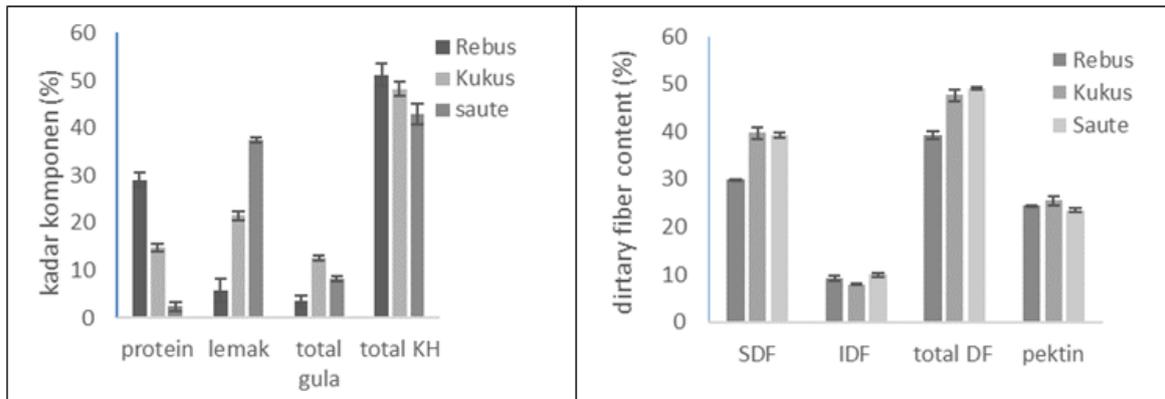
\*Mean from 6 repetitions

\*\* SD with superscripts which is different from each column differ significantly (p<0.05)

**Table 2.** Diabetic rats blood glucose level change for 4 weeks

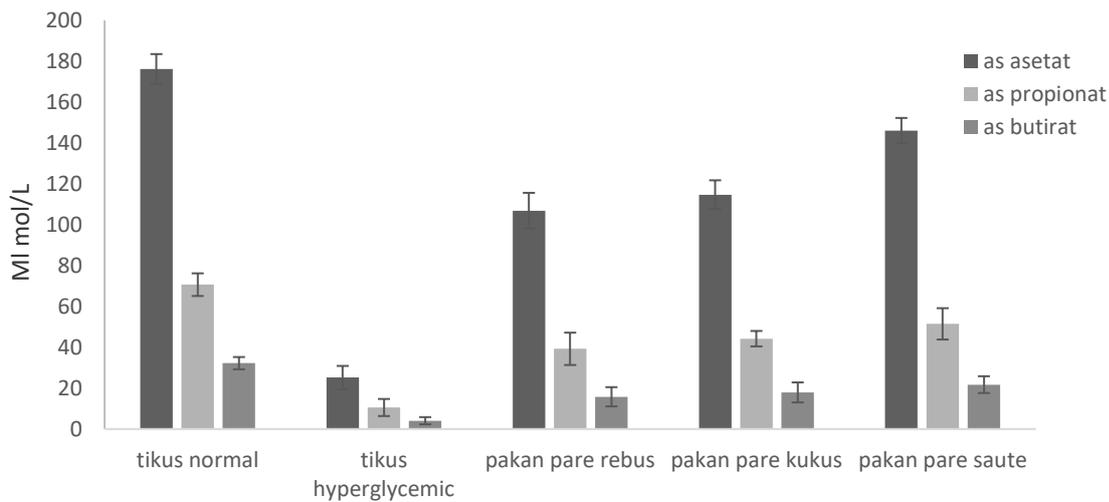
Food treatment	Blood glucose content (mg/dL)					Decrease/increase Blood glucose level (mg/dL)
	Week 0	1	2	3	4	
Control (-)	66,31a	66,94a	67,46a	68,58a	69,69a	3,38
Control (+)	218,47bc	220,15d	219,51d	222,53d	223,25d	4,78
Boiling treatment	216,67bc	194,34c	173,62c	133,55c	115,46c-	101,21
Steaming treatment	219,43c	186,89b	166,03b	126,48b	108,60b	-110,83
Sauting treatment	210,91b	187,62b	166,12b	126,84b	109,88b	-101,03

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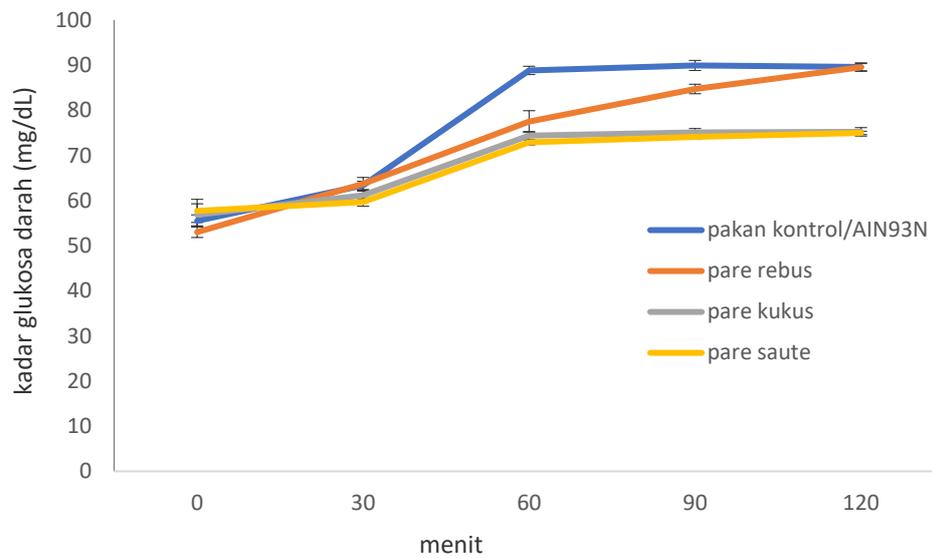


**Figure 1.** Component content of boiling, steaming and sautéing of bitter melon. (Left)

**Figure 2.** Dietary fiber content of boiling, steaming and sautéing of bitter melon. (Right)



**Figure 3.** Acetate acid concentration, propionate and butyrate acid from “*caecum Digesta*” rats with AIN 93M standard diet and bitter melon of boiling, steaming and sautéing treatment



**Figure 4.** The changes of rats' blood glucose level for Glucose Response Test with AIN 93M standard diet and bitter melon of boiling, steaming and sautéing treatment



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